REMARKS

Claim Objections:

2. Claims 9, 11-13, 15-19, and 46 are objected to under 37 CFR 1.75(c) as being in improper form. Applicants have amended the claims to correct the dependency of a multiply dependent claim on another multiply dependent claim. Specifically, Applicants have amended Claim 9 to depend on Claims 1-4 (excluding Claim 5) and adding a new dependent Claim 48 drawn to a chimeric gene comprising the isolated nucleic acid fragment of Claim 5 (depending on Claims 1-4) operably linked to suitable regulatory sequences. No improper multiply dependency remains.

Reconsideration of Claims 9, 11-13, 15-19, and 46, withdrawal of the objection, and prompt allowance of the claims is respectfully requested.

Patentability

2. Claims 1-5 and 23 stand rejected under 35 USC § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Applicants traverse the rejection and also have amended Claims 1-5 and deleted Claim 23.

The remaining rejected claims and specification supply far more than mere functional attributes. In addition to the specific sequences provided in the specification, Applicants have defined sequence variations that are also operable in the invention. These additional elements (i.e., stringent hybridization, a functional property, the two sequences, and the deposit of a biological material having a nitrilase activity) together supply the evidence of the Applicants' possession of the claimed invention.

Applicants have amended Claims 1 and 2 to specify the functional activity in the body of the claims. The criticized language (i.e., "encoding a substantial portion" and "substantially similar") has been deleted, even though the terms are defined in the specification and when the claims are read in whole, possession of the invention is clear. Applicants reserve the right to file continuing applications to this subject matter.

Reconsideration of Claims 1-5, withdrawal of the rejection, and prompt allowance of the claims is respectfully requested.

3. Claims 2 and 5 stand rejected under 35 USC § 112, second paragraph, for indefiniteness with regard to the percent identity parameter in the claims of 71 %.

Claim 2 as filed referred to a 71 % identity as determined by the described algorithm. The sequence comparison was performed relative to that of the *E. coli* SS1001 nitrilase sequence, yielding a percent identity of 70.732. Applicants can provide a statement on the default values used in determining the % identity limitation if this information may be included in the record and would be useful in the prosecution.

4. Claims 1-5 and 23 stand rejected under 35 USC § 102(b) as being anticipated by Kobayashi *et al.* (*Biochemistry* 31:9000-9007 (1992)).

A rejection for anticipation under 35 USC 102(b) requires that all elements of the claimed invention be found in the cited reference. Applicants strongly disagree that "Here, the only difference between the products is the claimed method of making". Applicants maintain that the composition of Kobayashi (the nucleic acid sequence that encodes for the *Rhodococcus rhodochrous* K22 nitrilase) and the compositions of Claims 1-5 and 23 (nucleic acid sequences that encode for the *Acidovorax facilis* 72W nitrilase) are not the same. This position is supported by the fact that the nitrilase enzyme encoded by the gene sequence reported by Kobayashi *et al.* has markedly different enzyme activities and temperature stability as compared to the nitrilase enzymes encoded by the nucleic acid fragments claimed in the present application.

Kobayashi *et al.* (*Tetrahedron* (1990) 46:5587-5590) reported on page 5589 that: "In the present study, the *Rhodococcus* K22 nitrilase was capable of hydrolyzing only one cyano group of aliphatic dinitriles to a carboxylic acid group." In this study, glutaronitrile was hydrolyzed quantitatively to 4-cyanobutyric acid (no detection of glutaric acid), and adiponitrile was quantitatively hydrolyzed to 5-cyanovaleric acid (no production of adipic acid). In contrast, Gavagan *et al.* (*J. Org. Chem.* 63:4792-4801 (1998)) reported that *Acidovorax facilis* 72W nitrilase (a nitrilase encoded by the nucleic acid fragments of the present application) hydrolyzed glutaronitrile to both 4-cyanobutyric acid (92 % yield) and glutaric acid (8 % yield), and hydrolyzed adiponitrile to produce 5-cyanovaleric acid in less than 50 % yield, with adipic acid being the major hydrolysis product.

In a separate publication, Kobayashi *et al.* (*J. Bacteriology* (1990) 4807-4815) report the substrate specificity of the *Rhodococcus rhodochrous* K22 nitrilase (page 4813, Table 3). Gavagan *et al.* (*Appl. Microbiol. Biotechnol.* (1999) 53: 654-659 in Table 1, page 656) compare the substrate specificity of the *Rhodococcus rhodochrous* K22 nitrilase with the substrate specificity of *Acidovorax facilis* 72W nitrilase, the data clearly showing that these two nitrilase enzymes have completely different substrate specificities. For example, the

relative % activity of succinonitrile and crotononitrile as substrates with K22 nitrilase are 271% and 100 %, respectively; in contrast, there is no detectable activity for conversion of crotononitrile with 72W nitrilase when compared to succinonitrile, which is a substrate. Similarly, the relative % activity for valeronitrile and fumaronitrile as substrates with K22 nitrilase are 27.4 % and 40.8 % respectively; in contrast, there is no detectable activity for conversion of valeronitrile with 72W nitrilase when compared to fumaronitrile, which is the most active substrate listed in Table 1 for hydrolysis by 72W nitrilase. It is clear from comparing the data in the two tables that the K22 nitrilase has a markedly different substrate specificity when compared to 72W nitrilase, and that unlike K22 nitrilase, the 72W nitrilase does not readily hydrolyze aliphatic mononitriles such as butyronitrile, valeronitrile, and capronitrile. Instead, the 72W nitrilase shows a clear preference for the hydrolysis of aliphatic dinitriles that is not exhibited by the K22 nitrilase.

Kobayashi *et al.* (*J. Bacteriology* (1990) 4807-4815) also report the temperature stability of *Rhodococcus rhodochrous* K22 nitrilase (page 4811, last paragraph). After preincubation at 50 °C for one hour, followed by assay at 25 °C, the K22 nitrilase had only 6.7 % of its initial activity. In marked contrast, Gavagan *et al.* (*Appl. Microbiol. Biotechnol.* (1999) 53: 654-659, page 655, last paragraph) report, that there was no loss of activity of 72W nitrilase after heating for one hour at 50 °C. Kobayashi *et al.* report a 28 % recovery of K22 nitrilase activity after preincubation for one hour at 45 °C, while Gavagan *et al.* report that the 72W nitrilase takes 14.6 days to lose 50 % of its activity at 45 °C. The temperature stabilities of these two nitrilases are markedly different.

The completely different characteristics (i.e., substrate specificities, thermal stabilities, and reactivities with the same substrates) that are reported for the nitrilases from *Rhodococcus rhodochrous* K22 and *Acidovorax facilis* 72W are clear evidence that different nucleic acid fragments encode each of the two nitrilases.

In the proceeding remarks, Applicants have discussed the substantial functional differences between the referenced and the claimed isolated nucleic acid fragments. These functional differences are evidence that the materials must be distinct compositions. Therefore, Applicants amend the rejected claims to further require that the claimed nucleic acid sequence encodes a polypeptide that hydrolyzes aliphatic dinitriles (or, alternatively hydrolyzes adiponitule to 1) 5-cyano valeric acid in less than 50% yield and 2) adipic acid).

The vague similarities of the Kobayashi nucleic acid sequence encoding K22 nitrilase to the claimed nucleic acid sequences cited by the examiner (where the cited K22 nucleic acid sequence has only a 71.274 % homology to SEQ ID NO: 5) do not prove that "the only

difference between the products is the claimed method of making" and are insufficient to uphold a rejection for anticipation. It is clear that the Kobayashi nucleic acid sequence is not identical to the claimed sequences, nor does the sequence encode a nitrilase activity with even remotely similar substrate specificity, regioselectivity, or thermal stability.

In light of the comments and amendments, Applicants respectfully request reconsideration of the claims, withdrawal of the rejection, and prompt allowance of Claims 1-5.

Allowable Subject Matter

5. Claims 10, 14, and 20 are objected to as being dependent upon a rejected base claim, "but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims."

Applicants respectfully suggest that these three claims are allowable as written and believe that this objection was unintended. Claims 10 and 20 are independent claims, drawn (as stated on page 8 of the Office Action) to specific, properly deposited biological materials neither taught nor suggested in the prior art. Claim 14 is dependent on independent Claim 10. Reconsideration of the Claims, withdrawal of the objection, and the prompt allowance of Claims 10, 14, and 20 is respectfully requested.

Additional Remarks

1. Applicants have further amended Claim 3 to delete reference to certain SEQ ID NOs: that do not describe an isolated nucleic acid fragment encoding a nitrilase enzyme. These were included in the originally filed claim in error.
2. Claims 21, 22, and 23 have been cancelled.

In light of the Remarks and amendments proposed herein, reconsideration of the Claims, withdrawal of the objection, and the prompt allowance of the claims under prosecution are respectfully requested.

Respectfully submitted,

LINDA AXAMETHY FLOYD
ATTORNEY FOR APPLICANTS
REGISTRATION NO. 33,692
TELEPHONE: 302-892-8112
FACSIMILE: 302-992-5374

Dated: 19 February 2003